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MONKEY ONKOGENIC VIRUS, CAUSING THE DEVELOPMENT OF TUMORS IN MAN

Following is the translation of an article by V. A. Parnes, Institute of Virology imeni D. I. Ivanovskogo, AMN USSR, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology), No 6, 1963, pages 643-649. It was submitted on 27 Jul 1963.

In recent years a number of works have appeared which have pointed out that many onkogonic viruses do not possess a strict species specificity and are capable of affecting animals of species which are very remote in a phylogenetic respect. The most striking example is the virus of Raus chicken sarcoma which is capable of affecting not only fowl, but also mammals - white mice and cotton rats, hamsters, rabbits, and guinea pigs [1, 5, 7-9]. The polyoma virus affects 6 different species of mammals [33, 41]. Also not strictly species specific are the viruses of mouse leucoses - Grossa, Graffi, Friend, Moloni, Mazurenko, and Rausher [6, 20, 21, 27, 28, 36]. The SV₄₀ monkey virus is capable of causing tumors in hamsters, rats, and Mastomys [3, 4, 13, 14, 17, 18, 34]. Just as the polyoma virus, when it is administered to the human organism it causes the development of specific antibodies [41], [24, 29, 42]. The SV₄₀ virus preserves its viability in the human organism and is discharged into the external environment with secretions and excreta [25, 29]. In a cell culture of man and in vitro the SV₄₀ virus displays a clearly expressed cytopathic effect [15, 38] and is able to cause the transformation of cells [13, 22, 31, 35, 37].

The question of the capacity of onkogonic viruses of animal origin to cause the development of tumors in man is exceedingly important both in a theoretical and practical respect in connection with the problem of the onkogonicity of virus vaccines.

Recently a new virus was isolated from monkeys (Yaba virus). It attracted attention to itself because it possesses the capacity to cause tumors both in monkeys and in persons which are infected with it. This is the first virus of animal origin in respect to which it has been proven that it is capable of causing tumors in man.

History of isolation of the virus. In 1957 in Western Africa, in Nigeria (Yaba) an "epidemic" of cutaneous tumors was described in a colony of monkeys. At first the tumor was revealed on the snout of an imported monkey. The monkeys imported into Yaba were Asiatic monkeys *Macaca mulatta* and they were kept in an open enclosure. Soon the same kind of tumors appeared on 20 imported Asian Rhesus monkeys which were kept in an enclosure neighboring the first one. Native West African monkeys in the same colony remained healthy. Apart from the Rhesus monkeys only one young monkey, a baboon, became ill. [12]

Tumors on the snout and body grew rapidly and often reached several centimeters in diameter. Subsequently they underwent reverse development. A histological investigation showed that the tumors have a mesenchymal origin. They consist of large pleomorphic cells, many of which contain large eosinophilic inclusion bodies in the cytoplasm. The presence of the inclusion bodies in the cells provoked the thought about the possibility of their viral nature. The nature of distribution of the disease among the monkeys supported this assumption.

For clearing up the question of the role of the virus in the development of cutaneous tumors in monkeys samples of the tumors were sent in a frozen state to the National Institute for Medical Investigations in London. At the Andrews Laboratory it was proven that tumors could be caused by filtered material [2, 11, 30].

The virus which caused the development of the tumors was attributed to the group of varicellous viruses. This is a large virus which passes through Gradocol membranes with a pore size of 0.65 μ . When filtrates from the tumors were administered to Rhesus monkeys tumorous nodules appeared on the animals. These, just as with spontaneously emerging tumors, initially grew, reached maximum sizes, and then as a rule underwent reverse development. The tumors were transplanted from monkey to monkey by means of intramuscular and subcutaneous injections. The nodules appeared in 5-20 days after the injection [2].

Morphology of lesions. The growth of tumors emanated out of histiocytes [39]. Usually after the subcutaneous or intracutaneous injection of the virus the local effects were expressed insignificantly. A neutrophil reaction is observed only during the first day. Histiocytes migrate to the site of administration of the virus. In 48 hours they are the predominant cell form here. By the third day the histiocytes begin to undergo noticeable changes - they increase in size and acquire a polygonal shape. The cell nuclei exceed by 2-3 times their normal sizes, their contours become oval or curved, and the chromatin is dense and irregularly distributed in the cell. The cell membrane is thickened. There is a noticeable increase in the number and size of nucleoli. Many cells are found in a state of mitotic division. Multiplying rapidly, they at first give a neoplastic nature to the formations. Already on the 4th or 5th day the changed histiocytes form compact nodules 2-3 mm in diameter, which palpate easily. During microscopic investigation of the nodules 3-4 mitoses are seen in a field of vision; there are no signs of necrosis. The epidermis over the tumor is unchanged. In the course of the 2nd week in the subcutaneous cellular tissue and the skin a tumor with the characteristic structure is formed. Its component cells are elongated with spindle-shaped nuclei and relatively barren protoplasm. They are disposed in the tumor in parallel clusters implanted between the capillaries, but not lying parallel to them

as this takes place in granulation tissue. The tissue of the tumor acquires the appearance of a woven trail. On the 9-13th day after infection, when the tumor is 5-10 mm in diameter, such "woven trails" are seen best of all. There are no nodules of a granulated nature with necrosis in the tumors [40]. The reticular network is not changed significantly during growth of the tumor. In three weeks the proliferation activity of the cells is reduced. In the tumor along with the spindle-shaped cells in mitosis there appear large degenerative nondividing cells with pyknotic nuclei. Reverse development of the tumors begins. The epidermis over the tumor is ulcerous and often bacterial contamination is observed. However, regression of the tumor can also take place with unaffected epidermis [39, 40]. Deposition of collagen during regression of the tumor does not take place and a scar is not formed. The tumors caused by the Yaba virus never have a fibrous nature and are different in this respect from viral fibroma of rabbits or deer. Regression of the tumor proceeded without central necrosis, as a process of tumor disintegration. The latter is connected with the in vivo manifested cytopathic action of Yaba virus. Immunological reactions of the organism do not play a deciding role here. Neutralizing and complement-fixing antibodies appear relatively rapidly in the serum of monkeys which were infected with the Yaba virus. Their level is high during the growth phase of the tumor. Cell immunity also is not an important factor in the regression of the tumor. Plasmatic cells and lymphocytes accumulate on the periphery of the tumor and remain here until the reverse development is sufficiently far off and the mass disintegration of tumorous cells is taking place.

Following intravenous administration of the Yaba virus the entire body of the monkey is covered with numerous fine spots; in subsequent days the number of lesions decreases, but those that remain continue to increase in size. Tumorous nodes are formed not only in the subcutaneous cellular tissue, but also in the lungs, heart, and skeletal musculature. Generalized hyperplasia of the tissue of the lymph nodes and spleen is noted. It takes place at the expense of growth of reticulo-endothelial elements. Tumorous cells are not detected in the tissue of these organs. Also there are none in the tissue of the brain and the salivary and sex glands. In all cases the tumors caused by the Yaba virus have the nature of histiocytoma. The numerous tumorous nodes which develop in monkeys following the intravenous administration of the virus cannot be viewed as metastasis. They do not have the properties of malignant neoplasms and are connected with the influence of Yaba virus on the tissue and not with the spreading of tumorous cells [40]. Following administration to monkeys of the Yaba virus with a Freund supplement the tumors may also develop in the liver, spleen, or kidneys.

A characteristic peculiarity of tumors caused by the Yaba virus is the presence of inclusions of two types [2, 40]. Minute inclusions appear on the third day. They are dense circular, sharply outlined, basophilic structures which are located near the nuclei.

Gradually they become all the more expressed and occupy a larger part of the cytoplasm, forcing the nucleus to the side. Somewhat later large inclusions appear. They are barely distinguishable from the cytoplasm and have irregular contours. They are more expressed in tumors which have undergone reverse development. The fine inclusions are probably aggregates of virus DNA. Mature virus particles are not revealed in them. On the other hand, in the large inclusions there are many typical virus particles. They also contain amorphous material which represents cellular detritus.

In later stages in the cells of tumors vacuoles and neutral fat appear.

Morphology of the virus. In size and form the virus is similar to the variolar viruses. In thin sections of cells which have been contaminated with the virus masses of oval or rounded particles can be seen under the electron microscope at low magnification. They have a high electron density and are located in the perinuclear zone.

At high magnification it is possible to see that many particles have the form of small bricks in which internal structures stand out which differ in their density. On the average the dimensions of the bricks are 200X280 mμ [30].

Particles of Yaba virus undergo the same stages of development as viruses of variola group [30]. But there is no immunological affinity between them [30, 26]. The yaba virus has no other common traits with the variolar viruses. Necroses of virus-contaminated cells are not characteristic and are observed only in late stages. The virus does not have an affinity for squamous epithelium, does not grow on chorio-allantoic membrane of fertilized eggs, does not cause lesions on the skin of a rabbit, and does not multiply in the organism of mice.

Multiplication of the virus in tissue cultures. In the first experiments on the cultivation of Yaba virus in a culture of monkey kidney [30] certain features in the multiplication of the virus were revealed. These could be used to judge its cytopathic effect, which was manifested in an increase in the size of the cells and intensive granulation of the protoplasm in the initial stages of cultivation of the tumorous suspensions and in the first and second passages in the same culture. In subsequent passages a change was noted in the nature of the cytopathic action of the virus. Multinuclear cells with sharply vacuolized protoplasm appeared in the culture. Following the administration of cultural material from the first - third passages to monkeys the development of tumors was observed regularly. Materials from cultures of subsequent passages were not active. Continuous cultures of Yaba virus could not be obtained in monkey kidneys. [30] In a HeLa culture and in a culture

of chick fibroblasts the virus did not multiply [30]. According to more recent data [10] the Yaba virus does not cause a cytopathic effect and does not multiply in a number of cell lines: Chang line of cells of human conjunctiva and liver, the skin of a human embryo Gray, D₆ and D₉₃ cells of human bone marrow of Serman and Shtalberg, intestinal cells of a human embryo Henley and McLa. Only in the Solk and Ward line of monkey heart cells was an insignificant increase in titer of the virus noted in a number of cases. It was determined by means of infection of monkeys with successive dilutions of cultural material. A cytopathic effect was not noted in a culture of monkey heart [10]. The authors also didn't note a cytopathic effect and multiplication of the virus in primary cultures of monkey kidney and mice embryos [10].

Experiments on the infection of monkeys. Since the virus could not be maintained in vitro a method was developed for titration of the virus in Rhesus monkeys. A tenfold dilution of virus suspension from 10^{-1} to 10^{-8} was applied to the shaved surface of the skin on the back. 10 days after infection with dilutions of 10^{-1} , 10^{-2} , and 10^{-3} the first tumorous nodules appeared. On the 55th day they reached maximum dimensions and necrotic changes appeared in them. By this time the first nodules appeared from dilutions of 10^{-4} and 10^{-5} , and by the 70th day also from a dilution of 10^{-6} . In the majority of tumors caused by the administration of higher concentrations of virus massive necroses were observed by this time. On the 80th day in the majority of tumorous nodes necroses and hemorrhages were noted. The animals lost weight, ate poorly, and generally were in poor condition. Subsequently they recovered, but in various sectors of the body secondary tumors often appeared. Sometimes from three to five infectious cycles were observed. New lesions developed in remote sectors of the body at approximately the same time that complete resorption of the old tumorous nodes was noted. In other cases after resorption of the tumor immunity developed in the monkeys; new tumors no longer appeared [10]. In the experiments of Grace and associates [19] the first tumors appeared on Rhesus monkeys at the site of administration of the virus approximately by the 7th day. They grew and reached maximum dimensions (4-6 cm) in 1-2 months, and then the rapid reverse development of the tumors began. In 2-3 weeks after infection neutralizing and complement fixing antibodies were noted in the blood of the animals [19]. The monkeys usually became immune to a subsequent administration of the virus.

Attempts were made to cause malignization of tumors in monkeys. For this purpose the skin of monkeys which had been infected with the virus was smeared daily with carcinogens (methylcholanthrene or dibenzanthracene) in various concentrations. Control animals were treated only with solvents of carcinogens - acetone or benzol. In monkeys which were treated with carcinogens malignization of tumors was not observed. In many cases regression of tumors began

in them even earlier than in animals of the control group. An analogous result was obtained during electrocautery of the central part of the tumor. This was carried out after 24 hours. Total irradiation of the monkeys (200 - 400 R) prior to infection with the virus and treatment of the animals with steroid hormones did not change the nature of the process. On monkeys which were infected with the Yaba virus experiments were set up for testing a number of antitumoral preparations (6-mercaptopurine, 5-fluorouracil, 5-fluorodeoxyuridine, 5-mercaptopuracil, and others). The drugs were administered daily to the animals subcutaneously, intraperitoneally, and intravenously in various doses. The development of the tumors was considered in the test and control groups of monkeys which had received similar doses of viral suspension. Indices of action for the drugs were the minimum doses of virus which caused the development of tumors, rate of development of the tumors, their maximum dimensions, and the time for the onset of regression. Based on all these indices no significant differences were noted between the control and test monkeys [10].

Immunity to the virus. With tumors caused by the Yaba virus the titers of antibodies in the blood were relatively high. A correlation is observed between the titers of complement fixing and neutralizing antibodies. Circulating antiviral antibodies are effective in respect to the prevention of tumorous growth, but do not have an effect on tumors which are already established. Animals with rapidly growing tumors and high titers of antibodies are resistant to the administration of a supplementary dose of virus. The circumstance that the rapid growth of tumors may take place with high titers of humoral antibodies indicates that the antibodies may not react with a virus which is found within the tumorous cells. Together with this they are capable of inactivating newly administered virus. The thought was expressed that in the organism of an animal an equilibrium is established between the amount of virus which is liberated from the damaged cells and the circulating antibodies [10]. Sometimes the virus is preserved in the cells, at the same time when the titer of antibodies in the blood is reduced below the level which prevents the spreading of the virus - then the infection of new sectors of the animal's body takes place; one more cycle of the disease begins. The connection of the cyclic nature of the disease only with a disruption of the balance between the antibodies circulating in the blood and the amount of virus liberated from the damaged cells has not received complete experimental confirmation. The administration of the virus to monkeys at the moment when after regression the titer of humoral antibodies in them was reduced to the initial level did not lead to the development of the disease [10]. In another experiment the administration of a supplementary dose of Yaba virus to monkeys with regressive tumors, but not displaying antibodies in the blood, caused the development of palpable tumors. The rapid increase of titers of antibodies in the blood was noted in the animals. The tumors reached a size of 0.5 - 1 cm in diameter [26]. A certain contradictory

nature in the results of the immunological experiments mainly indicates that other factors may interfere in the process (factors of tissue resistance, change in the state of the virus, etc.). A study of the dynamics of accumulation of antibodies in the blood of animals which were infected with the virus showed that the amount of antibodies in the blood increases rapidly and reaches a maximum on the 14-21st day. The antibodies remain at this level until the tumor is resorbed. After regression of the tumor the titers of antibodies is reduced or antibodies cannot be detected at all; they disappear from the blood stream. The same takes place after removal of the tumor. The duration of immunity during infection with Yaba virus is not great.

For clearing up the antigenic specificity of the Yaba virus cross experiments were set up with the vaccine virus and the virus of monkey pox [23, 32]. In monkeys which were immune to this virus typical lesions developed following the administration of Yaba virus to them. Synomolgus monkeys, which were resistant to the agent of infectious pustular dermatitis of monkeys (Orf-virus), were sensitive to the Yaba virus [26, 30].

Species specificity of the virus. For clearing up species specificity the virus was administered to newborn and adult dogs, cats, rabbits, guinea pigs, hamsters, rats (Wistar, Fischer 344), and mice (1CN/Na, C57, C3Ht). The material (homogenates or cell-less filtrates of the tumor) was administered to the test animals intravenously, subcutaneously, intraperitoneally, intranasally, and in the brain. No characteristic lesions appeared [16]. Only sometimes at the site of administration minute condensations were observed. These consisted of inflammatory elements.

Administration of virus filtrates into the vitelline sac or on the chorioallantoic membrane of developing chick embryos also did not lead to the multiplication of the virus. Material from these eggs did not cause tumors in monkeys [10, 19, 30].

Subcutaneous transplantation of cellular homogenates from tumors on Rhesus monkeys to other species of monkeys was successful in a number of cases. All these species of monkeys were then tested for susceptibility to the administration of cell-less viral material. Minute inflammatory nodules appeared on monkeys of many species, but they disappeared rapidly. The monkeys were admitted as susceptible to the virus only in the case that the lesions reached dimensions of no less than 0.5 cm in diameter, had the characteristic histological structure, did not resorb in the course of no less than 3 weeks, and during subinoculation with cellular homogenate caused the development of tumors in other monkeys. The virus caused tumors (histiocytoma) in Macaca mulatta (Rhesus), Macaca irus (Synomolgus), and Macaca nemistrinus. In green marmosets

(Cercopithecus aethiops) tumors developed after a lengthy latent period and reached maximum in 16-20 weeks after infection. In many species of African and South American monkeys the virus did not cause the development of tumors.

Epidemiology of the disease. For studying the paths of spreading of the disease monkeys which were infected with the virus were kept for 3 years in the same installation with 250 healthy noninfected monkeys. Contact outbreaks of infection were not observed. In another experiment infected and control animals were placed in the same cage. No illness was noted in the control animals /10/. It can be conjectured that the "outbreak" of the disease which was observed in 1957 in Yaba was connected with the spreading of the virus by an insect carrier.

Infection in man. During infection of monkeys with the virus one of the workers accidentally pricked his thumb with a needle which was contaminated with virus. At the site of the pricking an insignificant reddening appeared; it then passed away. For 4 months no signs of damage to the tissue were noted, but then on the finger at the site of the pricking a tumorous nodule appeared. It grew rapidly and soon reached 2 cm in diameter. The tumor was removed. Histologically it was no different than the tumors in monkeys. Growth of complement fixing antibodies to the virus was noted in the blood. Over a period of 2 years after removal of the nodule no manifestations of the disease were observed in this worker /10, 19/.

Cell-less filtrates from tumors of monkeys was administered to 6 volunteers with incurable cancer. After 5-7 days in all of them palpable nodules developed which grew slowly until they reached 2 cm in diameter. From this moment they began to resorb rapidly. The histological structure of the tumors from volunteers repeated exactly the structure of tumors in monkeys. Proliferative reactions to the virus in man were less expressed than in monkeys and resorption proceeded rapidly. The virus was isolated easily from the tumors which developed in the volunteers and was passaged serially. Suspensions and cell-less filtrates from tumorous nodules which were removed from the volunteers caused the characteristic tumors in other volunteers and in monkeys. Titration of the virus confirmed that it multiplied in the organism of man. Complement fixing antibodies were revealed in the blood of volunteers.

In general the titers of antibodies in man were lower than in monkeys /26/, but the amount of virus administered was also considerably less. Antibodies were revealed in volunteers in three successive intracutaneous passages. These were carried out both with cell-less filtrates and tumorous suspensions. Each material was administered to a volunteer in three sections of the arm.

Tumors developed in various periods (10-17 days) in all the sectors. In all cases it was possible to isolated the virus from them [26].

Thus it was proven that monkey tumorous virus can multiply and cause the growth of tumors in man.

The study of Yaba virus and the lesions caused by it began recently. Further study will bring new interesting data.

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